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Microbial Community Profile of a Lead Service Line Removed from a Drinking Water Distribution System▽

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A corroded lead service line was removed from a drinking water distribution system, and the microbial community was profiled using 16S rRNA gene techniques. This is the first report of the characterization of a biofilm on the surface of a corroded lead drinking water service line. The majority of phylotypes have been linked to heavy-metal-contaminated environments.

The U.S. Environmental Protection Agency's (U.S. EPA) Lead and Copper Rule established an action level for lead of 0.015 mg/liter in a 1-liter first-draw sample (16, 17, 18, 31, 32) at the consumer's tap. Lead release from drinking water distribution system (DWDS) materials is largely controlled by the solubility of lead-based solids (passivating solids) that form on lead materials (e.g., solder, lead pipes, brass fixtures, etc.). These lead-based solids include Pb(II) and Pb(IV) minerals. The most important passivating Pb(II) minerals include $Pb_3(CO_3)_2(OH)_2$ (hydrocerussite), $PbCO_3$ (cerussite), and $Pb_{10}(CO_3)_6(OH_6)O$ (plumbonacrite) (25). Aside from passivating solids, nonleaded solids and sorbed ions are often incorporated into the solids or scales that form on the surface of lead-based DWDS material.

Biofilms have been shown to influence the corrosion behavior of metals, including those used in drinking water pipes (6, 7, 9, 11, 48). For example, drinking water isolates of the genera *Agrobacterium*, *Acidovorax*, *Sphingomonas*, and *Micrococcus* have been reported to increase copper levels in drinking water pipes, with corrosion dependent on microbial activity (9, 10, 14, 23, 37). In other studies, *Rhodococcus*, *Stenotrophomonas*, and *Xanthomonas* have been shown to decrease copper levels by sorbing soluble copper in their exopolysaccharide (EPS) (2, 28, 47). Nitrification may also promote corrosion, as this process results in a pH drop which increases the solubility of most lead minerals commonly found on the surfaces of lead materials in DWDSs (48, 49).

Although researchers recognize that biofilms have the potential to impact metal release from DWDS materials, this conclusion is based largely on bench- or pilot-scale studies or speculation about the impact of microbial activity (e.g., local water quality changes, release of extracellular material, oxidant demand, etc.) on metal release. Furthermore, the identification of biofilm communities on materials removed from full-scale DWDSs by using molecular approaches is limited or nonexistent for some materials.

The goal of this study was to examine a corroded lead drinking water service line removed from the community's distribution system, survey the microbial community of a biofilm associated with lead corrosion by-products and deposits using molecular clone libraries, and discuss the relevance of identified microorganisms to lead corrosion and release. To our knowledge, an analysis of the microbial community of a biofilm associated with a lead service line removed from a real DWDS by molecular methods has never been reported.

In this study, a water utility in Illinois provided a lead service line for microbiological and mineralogical analysis. The service line was removed from the distribution system, immediately sealed under tap water to preserve the biofilm, and shipped overnight to the research team for analysis. The community's water treatment consisted of iron removal and lime softening (the finished water quality characteristics are presented in Table 1). The system historically maintained a free-chlorine residual despite having elevated ammonia levels (0.6 mg/liter) in the finished water; however, the utility recently (approximately 2 years ago) reduced the chlorine feed to the point of having no free-chlorine residual in the distribution system. This reduction was made to reduce disinfection by-products and resulted in chloramines as the primary disinfectant. The utility noted that a dramatic increase in lead levels in the distribution system coincided with the change in chlorine feed. Specifically, results obtained after the Lead and Copper Rule testing showed a 90th percentile lead value of 0.001 mg/liter prior to the disinfectant change, which increased to 0.0642 mg/liter the first monitoring period following the disinfectant change.

By use of an aseptic technique, a single pipe section was cut in half with a band saw, and approximately 2.80 cm^2 of corrosion by-products and deposits was scraped to a depth of approximately 350 μm using sterile spatulas (32). Nucleic acid was extracted using the MasterPure DNA kit (EpiCentre Biotechnologies, Madison, WI) in accordance with the manufacturer's protocol. Nearly full-length 16S sequences (~1,400 bp) were PCR amplified using the forward primer 5′-GTTTGATCCTGGCTCAG-3′ and the reverse primer 5′-ACGGYTACCTTGTTACGACTT-3′ and subsequently cloned via a TOPO TA cloning kit (Invitrogen, Carlsbad, CA). The forward amplicon (~800 bp) was sequenced and identified using the BLAST feature of the GenBank database. The clone library was screened for chimeras using Bellerophon. Alignments were made using ClustalW; phylogenetic comparisons were made using the neighbor-joining algorithm with a maximum

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TABLE 1. Water quality

Characteristic[a]	Value
Analyte	
Al, mg/liter	0.004
As, mg/liter	0.00545
Ba, mg/liter	0.008
Ca, mg/liter	7.007
Cu, mg/liter	<0.001
Fe, mg/liter	0.0177
K, mg/liter	2.034
Mg, mg/liter	9.0245
Mn, mg/liter	0.0014
Na, mg/liter	40.465
NH_3, mg N/liter	0.56
Ni, mg/liter	<0.001
NO_2^-, mg N/liter	0.036
NO_3^-, mg N/liter	0.0995
Pb, mg/liter	<0.002
PO_4, mg/liter	0.0155
P, mg/liter	0.01495
Si, mg/liter	5.6875
Sr, mg/liter	0.0419
S, mg/liter	0.20275
TALK, mg $CaCO_3$/liter	132.88
TOC, mg C/liter	3.28
V, mg/liter	<0.001
Zn, mg/liter	<0.0005
pH, units	8.63[b]
DO, mg O_2/liter	7.57[b]
Temp, °C	24.2[b]
Free Cl_2, mg Cl_2/liter	1.23[b]

[a] TOC, total organic carbon; TALK, total alkalinity; DO, dissolved oxygen.
[b] Average result for five samples taken from the distribution system 1 year after the pipe was removed.

composite likelihood model assuming pairwise deletion in MEGA 4 (Biodesign Institute, Tempe, AZ) (45). The bootstrap consensus tree was generated using 5,000 bootstrap repetitions and is presented in Fig. 1. Operational taxonomic units (OTUs) are defined by a sequence similarity of >97% using Sequencher (Gene Codes Corporation, Ann Arbor, MI).

A total of 244 sequences were used for library construction and phylogenetic analysis. Overall, five phyla were represented (Table 2) and are graphically displayed as a phylogenetic tree (Fig. 1). The Chao-1 richness estimator was higher (42) than the number of observed OTUs (28), indicating that an increase in sampling would help describe the diversity. The largest number of sequences were closely related to the betaproteobacterial genus *Massilia*, accounting for over 55% of the clones. After the betaproteobacteria, the phylum *Firmicutes* accounted for 10 OTUs, with the three genera *Bacillus*, *Paenibacillus*, and *Exiguobacterium* represented. The alphaproteobacteria contributed the greatest diversity to the clone library, with 9 OTUs representing 9 unique genera. These 9 OTUs comprised four singleton (sequences represented only once) OTUs. The phyla *Deinococcus-Thermus*, *Bacteriodetes*, and *Actinobacteria* contributed 5 OTUs, collectively representing 4.5% of library clones.

Members of the genera *Bacillus*, *Sphingomonas*, *Arthrobacter*, *Brevundimonas*, and *Microbacterium* have all been previously identified as heavy-metal-tolerant bacteria. Studies have relied upon both culture- and non-culture-based methods to draw these conclusions. Specifically, species within *Bacillus* have been shown to produce a metallothionein-like protein which deactivates and accumulates lead intracellularly (19). Abou-Shanab et al (1) reported the MICs for diverse species of *Bacillus*, *Microbacterium*, *Sphingomonas*, *Arthrobacter*, and *Paenibacillus* to be in the range of 10 to 15 mM lead (2 to 3 g/liter). MICs for divergent methylotrophic bacteria and *Arthrobacter* were reported to be above 4.8 mM, but no upper value was given (12). Members of the genus *Massilia* have been

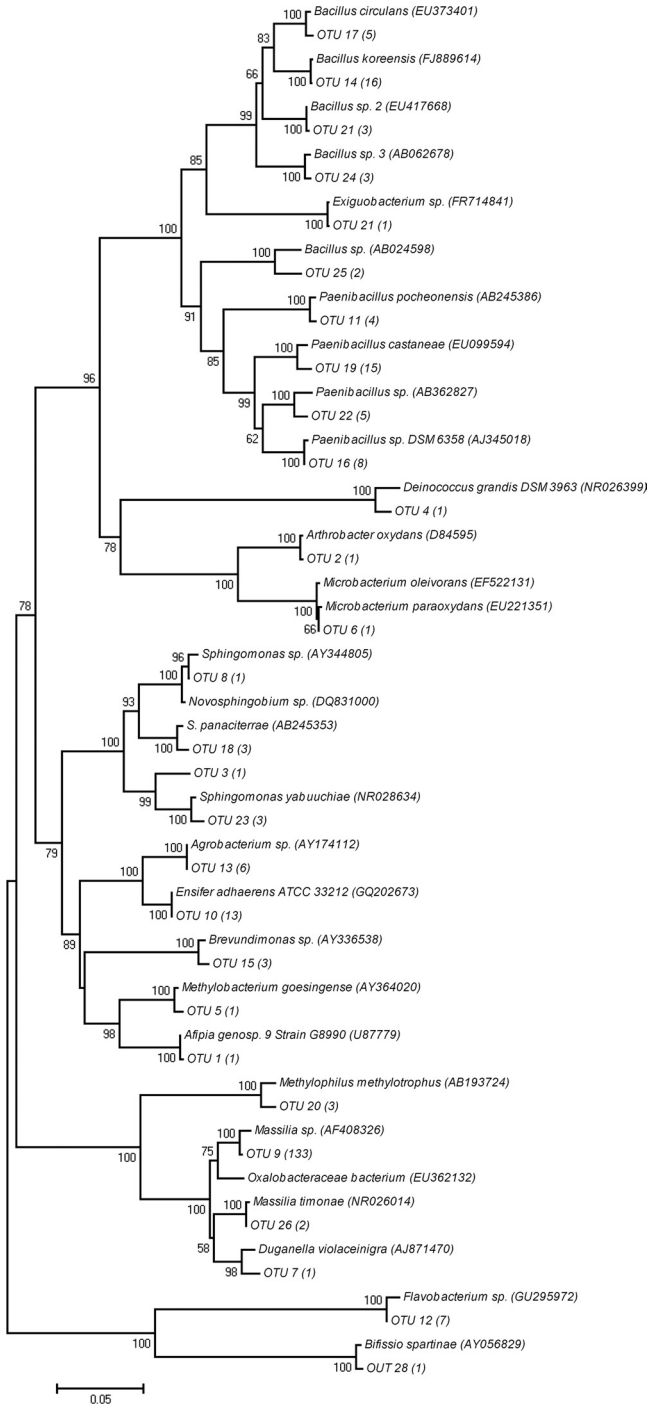

FIG. 1. Unrooted neighbor-joining tree of clone library, partial-length, 16S rRNA gene sequences.

TABLE 2. Clone library member identification and frequency

Closest relative in GenBank (accession no.)	No. of OTUs	Similarity (% coverage/ % identity)	No. of sequences	Phylum classification	% of library
Massilia timonae (NR026014)	26	100/99	2	*Proteobacteria*	70.1
Massilia sp. (AF408326)	9	100/99	133		
Oxalobacteraceae bacterium (EU362132)		100/97			
Methylophilus methylotrophus (AB193724)	20	100/98	3		
Duganella violaceinigra (AJ871470)	7	100/98	1		
Sphingomonas sp. (AY344805)	8	100/99	1		
Novosphingobium sp. (DQ831000)		100/99			
Sphingopyxis panaciterrae (AB245353)	18	100/99	3		
Sphingomonas yabuuchiae (NR028634)	23/3	100/99	3/1		
Agrobacterium sp. (AY174112)	13	100/100	6		
Ensifer adhaerens ATCC 33212 (GQ202673)	10	100/100	13		
Brevundimonas sp. (AY336538)	15	100/99	3		
Methylobacterium goesingense (AY364020)	5	100/99	1		
Afipia genosp. 9 strain G8990 (U87779)	1	100/99	1		
Paenibacillus sp. (AB362827)	22	100/98	5	*Firmicutes*	25.4
Paenibacillus sp. DSM 6358 (AJ345018)	16	100/99	8		
Paenibacillus pocheonensis (AB245386)	11	100/99	4		
Paenibacillus castaneae (EU099594)	19	100/98	15		
Bacillus circulans (EU373401)	17	100/99	5		
Bacillus sp. (AB024598)	25	100/96	2		
Bacillus sp. 2 (EU417668)	21	100/99	3		
Bacillus sp. 3 (AB062678)	24	100/99	3		
Bacillus koreensis (FJ889614)	14	100/99	16		
Exiguobacterium sp. (FR714841)	27	100/99	1		
Deinococcus grandis DSM 3963 (NR026399)	4	100/97	1	*Deinococcus-Thermus*	0.4
Microbacterium paraoxydans (EU221351)	6	100/99	1	*Actinobacteria*	0.8
Microbacterium oleivorans (EF522131)		100/99			
Arthrobacter oxydans (D84595)	2	100/99	1		
Flavobacterium sp. (GU295972)	12	100/99	7	*Bacteroidetes*	3.3
Bifissio spartinae (AY056829)	28	100/99	1		

isolated from both drinking water and heavy-metal-contaminated soils, though the details about the contaminating heavy metals have not been reported (21, 50).

It was interesting to note the presence of *Deinococcus*. Suresh et al (44) reported on a species of *Deinococcus* that was resistant to the metalloid arsenic. A review of the literature has indicated that resistance to one heavy metal is generally correlated to multiple-heavy-metal resistance (13, 27, 34, 41, 46).

Ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) were not detected in the biofilm sample via PCR despite the presence of ammonia in the source and finished waters. Matrix spikes using positive-control DNA and ammonia monooxygenase subunit A (*amoA*)-specific DNA primers were positive, suggesting that the DNA extract matrix did not have an inhibitory effect on the PCR. PCR tests on nucleic acid recovered from fire hydrant flush samples collected in the same distribution system did test positive for AOB. AOB have been associated with lead release, and the mechanism has been attributed to the drop in pH during the nitrification process (48, 49).

These results showed that biofilm-forming organisms whose 16S sequences are similar to those of organisms identified as heavy-metal-resistant bacteria, many of which are known to accumulate heavy metals, were present on the surface of the lead service line. Given the nature and reported activity of the bacteria identified in this study, the biofilm can potentially be tied to lead release in the distribution system. We stress the necessity of culture-based identification both to elucidate lead resistance mechanisms and to support the idea that a selective pressure from the pipe material is driving the composition of the community. Microbial heavy-metal resistance, primarily in soils, is a well-researched field. Moreover, molecular characterization and isolation of microorganisms resistant to heavy metals are abundant. The common mechanisms of heavy metal resistance include ATPase efflux, oxidation/reduction, accumulation or immobilization in exopolysaccharides, precipitation, intracellular sequestration, and volatilization by the addition of methyl or ethyl groups (8, 15, 33, 36, 39, 40, 42, 43). Specifically, extracellular polysaccharides from *Paenibacillus* species have been used as a biosorbent for lead, sorbing over 300 mg Pb/g (29, 30). Many studies have documented lead toxicity as low (with higher MIC levels) compared to that of heavy metals such as Co, Cd, As, Cu, and Zn (13, 27, 34, 41, 46). Lead is toxic to cells because it damages DNA, disrupts membranes, and inactivates proteins (5, 20, 41). Lead also exerts a pressure on the microbial community, lowering total viable counts (3, 4, 22, 35, 38).

Lastly, it is worthwhile to note that a detailed mineralogical analysis of the pipe wall showed that Pb(IV) was present (32). A high and consistent oxidation-reduction potential (ORP) in the drinking water distribution system and at the pipe wall and the consistent presence of free chlorine are necessary to maintain a Pb(IV) mineral (24, 25, 26). Maybe not so surprising, despite evidence that high and consistent levels of chlorine have been present at the pipe surface, a biofilm is still present and presumably active.

Lead release from drinking water distribution system materials is attributed primarily to corrosion, mineral solubility, and

particle mobility and driven by water quality factors. The activity and properties of biofilm are also thought to contribute to the corrosion and release of metals from drinking water distribution system materials, including lead. This study was the first to identify a biofilm isolated from the surface of a lead service line removed from a drinking water distribution system. The analysis revealed the presence of a group of bacteria that included bacteria previously reported to exist in heavy-metal-contaminated environments and known to interact with heavy metals, including lead. The findings of this work confirm the conclusion that a biofilm can be associated with the surface of corroding lead surfaces in drinking water distribution systems. Furthermore, though we acknowledge that phylogeny does not infer physiology, a mechanism(s) by which such a biofilm can affect the mobilization of lead has been described for genera matching those found here. It is important to note that physiology is divergent and specific to the strain level and further analysis is required, including the isolation of pure cultures and molecular work such as pyrosequencing and metabolomic analysis. The control of biofilm activity must be considered when developing lead control strategies.

Nucleotide sequence accession numbers. Sequences generated in this study have been deposited in GenBank under accession numbers HM998727 to HM998752.

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REFERENCES

1. **Abou-Shanab, R. A. I., P. van Berkum, and J. S. Angle.** 2007. Heavy metal resistance and genotypic analysis of metal resistance genes in gram-positive and gram-negative bacteria present in Ni-rich serpentine soil and in the rhizosphere of Alyssum murale. Chemosphere **68:**360–367.
2. **Arens, P., G. J. Tuschewitzki, M. Wollmann, H. Follner, and H. Jocabi.** 1995. Indicators for microbiologically induced corrosion of copper pipes in a cold-water plumbing system. Zentralbl. Hyg. Umweltmed. **196:**444–454.
3. **Atuanya, E. I., and E. O. Oseghe.** 2006. Lead contamination and microbial lead tolerance in soils at major road junctions in Benin City. J. Appl. Sci. Environ. Manage. **10:**99–104.
4. **Benka-Coker, M. O.** 1991. Studies of the effect of crude oil and heavy metal contamination of soil and water on the microbial populations at the Kokori Flare Site, Bendel State, Nigeria. Ph.D. thesis. University of Benin, Benin City, Nigeria.
5. **Beveridge, T. J., and R. J. Doyle.** 1989. *In* Metal ions and bacteria. John Wiley and Sons, Inc., New York, NY.
6. **Bremer, P. J., and G. G. Geesey.** 1991. Laboratory-based model of microbiologically induced corrosion of copper. Appl. Environ. Microbiol. **57:**1956–1962.
7. **Bremer, P. J., B. J. Webster, and D. B. Wells.** 2001. Biocorrosion of copper in potable water. J. Am. Water Works Assoc. **93:**82–91.
8. **Collard, J. M., et al.** 1994. Plasmids for heavy metal resistance in *Alcaligenes eutrophus* CH34: mechanisms and applications. FEMS Microbiol. Rev. **14:** 405–414.
9. **Critchley, M. M., N. J. Cromar, N. McClure, and H. J. Fallowfield.** 2001. The effect of distribution system bacterial biofilms on copper concentrations in drinking water. Water Sci. Technol. Water Supply **1:**247–252.
10. **Critchley, M. M., R. Pasetto, and R. J. O'Halloran.** 2004. Microbiological influences in 'blue water' copper corrosion. J. Appl. Microbiol. **97:**590–597.
11. **Davidson, D., B. Beheshti, and M. W. Mittelman.** 1996. Effects of *Arthrobacter* sp., *Acidovorax delafieldii*, and *Bacillus megaterium* colonisation on copper solvency in a laboratory reactor. Biofouling **9:**4.
12. **De Marco, P., C. C. Pacheco, A. R. Figueiredo, and P. Moradas-Ferreira.** 2004. Novel pollutant-resistant methylotrophic bacteria for use in bioremediation. FEMS Microbiol. Lett. **234:**75–80.
13. **Dressler, C., U. Kües, D. H. Nies, and B. Friedrich.** 1991. Determinants encoding resistance to several heavy metals in newly isolated copper-resistant bacteria. Appl. Environ. Microbiol. **57:**3079–3085.
14. **Dutkiewicz, C., and H. Fallowfield.** 1998. Assessment of microbial involvement in the elevation of copper levels in drinking water. Appl. Microbiol. **85:**597–602.
15. **Ekundayo, E. O., and K. Killham.** 2001. Lead Solubilization and Accumulaiton by Two Strains of Pseudomonas Obtained from a Contaminated Alfisol's Effluent in Southwestern Nigeria. Environ. Monit. Assess. **71:**243–254.
16. **Federal Register.** 1991. Drinking water regulations; Maximum contaminant level goals and national primary drinking water regulations for lead and copper, final rule; correction. CFR 40, parts 141 and 142. Fed. Regist. **56:**32112.
17. **Federal Register.** 1991. Maximum contaminant level goals and national primary drinking water regulations for lead and copper, final rule. Fed. Regist. **56:**26460.
18. **Federal Register.** 1992. Drinking water regulations: Maximum contaminant level goals and national primary drinking water regulations for lead and copper, final rule; correcting amendments. CFR 40, parts 141 and 142. Fed. Regist. **57:**28785.
19. **Gadd, G. M.** 1990. Heavy metal accumulation by bacteria and other microorganisms. Cell. Mol. Life Sci. **46:**834–840.
20. **Gadd, G. M.** 1992. Microbial control of heavy metal pollution, p. 59–88. *In* J. C. Fry, G. M. Gadd, R. A. Herbert, C. W Jones, and I. A. Watson-Craik (ed.), Microbial control of pollution. Cambridge Press, Cambridge, MA.
21. **Gallego, V., C. Sanchez-Porro, M. Teresa Garcia, and A. Ventosa.** 2006. *Massilia aurea* sp. nov., isolated from drinking water. Int. J. Syst. Evol. Microbiol. **56:**2449–2453.
22. **Hughes, M. N., and R. K. R. K. Poole.** 1989. Metal and microorganisms, p. 1–37. Chapman and Hall, London, England.
23. **LeChevallier, M. W., T. M. Babcock, and R. G. Lee.** 1987. Examination and characterization of distribution system biofilms. Appl. Environ. Microbiol. **53:**2714–2724.
24. **Liu, H., G. V. Korshin, and J. F. Ferguson.** 2008. Investigation of the kinetics and mechanisms of the oxidation of cerussite and hydrocerussite by chlorine. Environ. Sci. Technol. **42:**3241–3247.
25. **Lytle, D. A., C. White, M. N. Nadagouda, and A. Worrall.** 2009. Crystal and morphological phase transformation of Pb(II) to Pb(IV) in chlorinated water. J. Hazard Mater. **165:**1234–1238.
26. **Lytle, D. A., and M. R. Schock.** 2005. Formation of Pb(IV) oxides in chlorinated water. J. Am. Water Works Assoc. **97:**102–114.
27. **Mergeay, M., et al.** 1985. *Alcaligenes eutrophus* CH34 is a facultative chemolithotroph with plasmid-bound resistance to heavy metals. J. Bacteriol. **162:** 328–334.
28. **Mittelman, M. W., and G. G. Geesey.** 1985. Copper-binding characteristics of exopolymers from a freshwater-sediment bacterium. Appl. Environ. Microbiol. **49:**846–851.
29. **Morillo, J., M. Aguilera, A. Ramos-Cormenzana, and M. Monteoliva-Sánchez.** 2006. Production of a metal-binding exopolysaccharide by *Paenibacillus jamilae*; using two-phase olive-mill waste as fermentation substrate. Curr. Microbiol. **53:**189–193.
30. **Morillo Pérez, J., et al.** 2008. Biosorption of heavy metals by the exopolysaccharide produced by *Paenibacillus jamilae*. World J. Microbiol. Biotechnol. **24:**2699–2704.
31. **Morse, D. L., W. N. Watson, J. Housworth, L. E. Witherell, and P. J. Landrigan.** 1979. Exposure of children to lead in drinking water. Am. J. Public Health **69:**711–712.
32. **Nadagouda, M. N., C. White, and D. Lytle.** 2011. Lead pipe scale analysis using broad-beam argon ion milling to elucidate drinking water corrosion. Microsc. Microanal. **17:**284–291.
33. **Nies, D. H.** 1999. Microbial heavy-metal resistance. Appl. Microbial. Biotechnol. **51:**730–750.
34. **Piotrowska-Seget, Z., M. Cycon, and J. Kozdroj.** 2005. Metal-tolerant bacteria occuring in heavily polluted soil and mine spoil. Appl. Soil Ecol. **28:** 237–246.
35. **Reber, H. H.** 1992. Simultaneous estimates of the diversity and degradative capability of heavy-metal-affected soil bacterial communities. Biol. Fert. Soils **13:**181–186.
36. **Rensing, C., Y. Sun, B. Mitra, and B. P. Rosen.** 1998. Pb(II)-translocating P-type ATPases. J. Biol. Chem. **273:**32614–32617.
37. **Reyes, A., M. V. Letelier, R. De la Iglesia, B. Gonzalez, and G. Lagos.** 2008. Microbiologically induced corrosion of copper pipes in low-pH water. Int. Biodeterior. Biodegradation **61:**135–141.
38. **Roane, T. M., and S. T. Kellogg.** 1996. Characterization of bacterial communities in metal-contaminated soils. Can. J. Microbiol. **42:**593–603.
39. **Roane, T. M.** 1999. Lead resistance in two bacterial isolates from heavy metal-contaminated soils. Microb. Ecol. **37:**218–224.
40. **Salmassi, T. M., et al.** 2002. Oxidation of arsenite by Agrobacterium albertimagni, AOL15, sp. nov., isolated from Hot Creek, California. Geomicrobiol. J. **19:**53–66.
41. **Schmidt, T., and H. G. Schlegel.** 1994. Combined nickel-cobalt-cadmium

resistance encoded by the *ncc* locus of *Alcaligenes xylosoxidans* 31A. J. Bacteriol. **176:**7045–7054.
42. **Sharma, R., C. Rensing, B. P. Rosen, and B. Mitra.** 2000. The ATP hydrolytic activity of purified ZntA, a Pb(II)/Cd(II)/Zn(II)-translocating ATPase from Escherichia coli. J. Biol. Chem. **275:**3873–3878.
43. **Silver, S.** 1988. Genes for all metals—a bacterial view of the periodic table. J. Indust. Microbiol. Biotechnol. **20:**1–12.
44. **Suresh, K., G. S. N. Reddy, S. Sengupta, and S. Shivaji.** 2004. *Deinococcus indicus* sp. nov., an arsenic-resistant bacterium from an aquifer in West Bengal, India. Int. J. Syst. Evol. Microbiol. **54:**457–461.
45. **Tamura, K., J. Dudley, M. Nei, and S. Kumar.** 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24:**1596–1599.
46. **Trajanovska, S., M. L. Britz, and M. Bhave.** 1997. Detection of heavy metal ion resistance genes in gram-positive and gram-negative bacteria isolated from a lead-contaminated site. Biodegradation **8:**113–124.
47. **White, D. C., S. D. Sutton, and D. B. Ringelberg.** 1996. The genus *Sphingomonas*: physiology and ecology. Curr. Opin. Biotechnol. **7:**301–306.
48. **Zhang, Y., A. Griffin, and M. Edwards.** 2008. Nitrification in premise plumbing: role of phosphate, pH and pipe corrosion. Environ. Sci. Technol. **42:** 4280–4284.
49. **Zhang, Y., et al.** 2009. Lead contamination of potable water due to nitrification. Environ. Sci. Technol. **43:**1890–1895.
50. **Zhang, Y. Q., et al.** 2006. *Massilia dura* sp. nov., *Massilia albidiflava* sp. nov., *Massilia plicata* sp. nov. and *Massilia lutea* sp. nov., isolated from soils in China. Int. J. Syst. Evol. Microbiol. **56:**459–463.